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Molecular Weight of Renin Determined by Sephadex G-200 Gelfiltration

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The molecular weight of renin has been determined by the use of the correlation between the elution volume from Sephadex G-200 and the molecular weight of three reference proteins, γ_G-globulin, human serum albumin, and Bence-Jones protein. A mean value of 43 000 was obtained.

As shown by several authors [1,16] gelfiltration on Sephadex may be used for molecular weight determination of proteins, if suitable reference proteins of known molecular weight are run on the same columns. This is due to the sieving effect of Sephadex, separating the molecules according to volume,[13] so that the proteins emerge in the order of decreasing molecular size from the column. The error in the molecular weight thus determined is in most cases less than 10 %, with certain exceptions.

In a preliminary report,[7] the results obtained by gelfiltration pointed toward a molecular weight for renin between 42 000 and 49 000. In the present investigation, these studies have been extended. Bence-Jones protein has been used as the main reference protein because the molecular weight of the dimer of this protein [14] is only little different from the one estimated previously for renin.

EXPERIMENTAL

Gelfiltration on Sephadex G-200 was performed in 0.1 M TRIS-buffer pH 8.0, M NaCl at 4°C on columns with a diameter of 2 cm and a $V_i + V_o$ (V_i being the internal aqueous volume of the Sephadex grains, and V_o being the outer volume) of about 350 ml as determined with ^{22}NaCl. Fractions of about 2 ml were collected by an automatic fraction-collector and protein concentrations determined by spectrophotometric measurement at 280 nm in a Beckman DU spectrophotometer or at 253.7 nm by an Uvicord continuous recorder (LKB, Stockholm, Sweden) during the gelfiltration experiments. The equivalent height of a theoretical plate [2] (E. H. T. P.) calculated from E. H. T. P. $= l/N$, and $N = 8(V_e/\beta)^2$, varied between 0.1 and 0.33 cm. l is the lenght of the column, N the number of theoretical plates, β the width of elution curve at the height $C_{max}/2.72$, and V_e the elution volume for the component studied.

^{125}I-Labelled Bence-Jones proteins of type lambda, were kindly supplied by Dr. Karsten Jensen, Department of Clinical Biochemistry, Bispebjerg Hospital.[6] Human serum albumin (Behringwerke, Marburg, Germany) and γ_G-globulin, purified by DEAE-Sephadex chromatography, were labelled with ^{131}I and ^{125}I, respectively, by the iodine monochloride method.[9] The purity of the labelled γ_G-globulin, albumin and Bence-Jones proteins was controlled by autoradiography of immunoelectrophoretic slides and of paper-electropherograms for these proteins. Also by gelfiltration on Sephadex G-200 only one radioactive peak appeared in each case. ^{131}I- and ^{125}I-activity was measured in a well-type scintillation counter with a pulse-height analyzer (Tracerlab.).

Goldblatt-renin, step V [5] (Biochemical Nutritional Company) or hog renin purified to step III (Crude enzyme) [8] was mixed with an amount of each of the labelled proteins ranging from 10^{-5} — 10^{-7} g, corresponding to about 50 000 cpm and 300 μl normal human serum to a total volume of 500 to 700 μl. The mixture was applied to the Sephadex G-200 column in 20 % sucrose. The recovery of renin varied between 100 and 120%.

Renin activity was estimated by the method of Skeggs, Kahn and Marsh.[15] One rat unit was refined as 1/40 of one Goldblatt unit. Goldblatt renin step V was used as a standard.

The molecular weight of renin was estimated from the correlation between the logarithm of molecular weight for the reference proteins and their elution volume (V_e).[1]

RESULTS

In all gelfiltration experiments the peak of renin activity emerged at the site or a few ml after the peak for the appearance of the Bence-Jones proteins (Table 1 and Fig. 1). With the Bence-Jones protein "F" the plot of the logarithm of the molecular weight *versus* the elution volume (Fig. 2) gave values between 42 000 and 44 000 with a mean of 43 000, while values of 38000 to

Table 1. Data on Sephadex G-200 gelfiltration of renin.

Expt. No.	Renin applied to column (1) (rat units)	Elution volume (ml)				Molecular weight estimated for renin
		^{125}I-γ_G-globulin (3) (M.W. 150000)	^{131}I-γ_G-albumin (4) (M.W. 69000)	^{125}I-Bence-Jones (2) and (3) (M.W. 44000)	Renin	
1	1500	196.3	243.9	274.9	279	42000
2	3000	192.2	238.7	268.7	269.7	43000
3	6000	175.2	225.6	259.2	261.6	43000
4	6000	180	228	264	261.6	44000
5	6000	177.6	225.6	261.6	264	43000
6	15000	194.4	248.4	284.4	286.8	43000
7	15000	202.8	261.6	297.6	297.6	44000
8	15000	193.6	243.8	275.3	277.2	43000
9	6000	195	241	265	270	40000
10	6000	176	221	252	262	38000
11	3000	166	212	237	242.7	40000

1. Exp. 1 — 5, 9, 10, and 11: Goldblatt renin (N.B.C.). Exp. 6, 7, and 8: Crude enzyme (Rubin & Kemp [8]).
2. Exp. 1—8: Bence-Jones "F". Exp. 9—11: Bence-Jones "M".
3. From reference 14.
4. From reference 10.

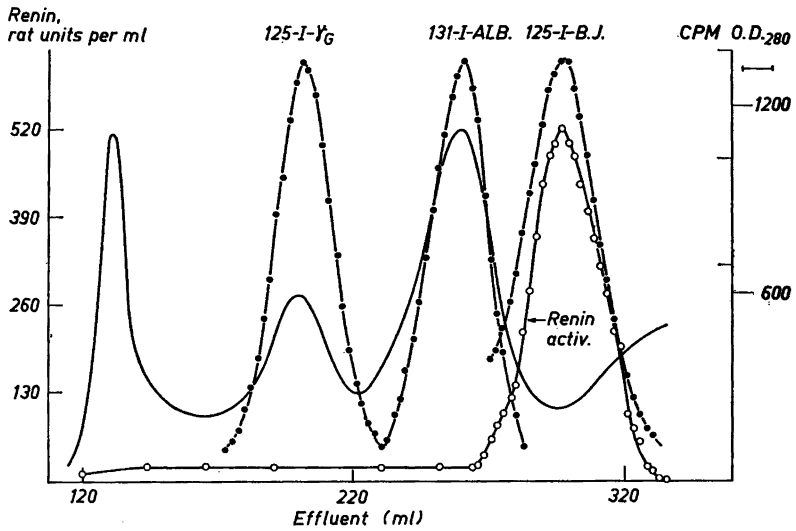

Fig. 1. G-200 Sephadex gelfiltration of a mixture of human serum (D_{280}:———), renin O———O), ^{125}I-labelled γ_G-globulin, ^{131}I-labelled albumin and ^{125}I-labelled Bence-Jones protein "F" (●———●).

40 000 were obtained with Bence-Jones protein "M". When decreasing amounts of renin were applied to the columns, in order to extrapolate to zero concentration, no significant change occurred in the molecular weights estimated (Fig. 3).

DISCUSSION

It is well established that molecular weight determinations for proteins by Sephadex gelfiltration in most cases agree with the values obtained by classical physico-chemical methods; for Sephadex G-200 the range for its use extends between about 10 000 and 300 000.

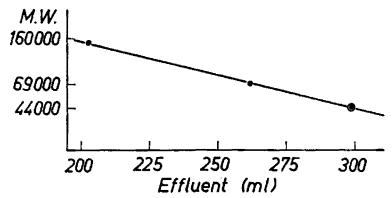

Fig. 2. Plot of the molecular weight for γ_G-globulin (15 0000), albumin monomer (69 000) and Bence-Jones protein "F" dimer (44 000), on a logarithmic scale *versus* the elution volumes for these proteins and for renin.

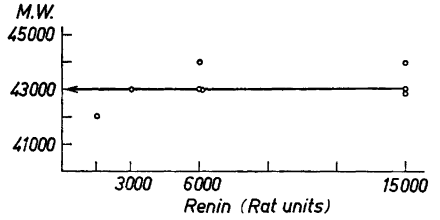

Fig. 3. Plot of the estimated molecular weight for renin *versus* the amount of renin applied to the Sephadex G-200 column.

Disagreement has been found in experiments with some aromatic components [12,3] and with the basic proteins [4] because of adsorption to the gel, while shape factors for the proteins usually have no influence; however, some proteins as, *e.g.*, glutamate dehydrogenase [1] or hemoglobin [16] dissociate when decreasing amounts are used, resulting in a reduction of the molecular weight estimated.

In the present study it appeared that the molecular weight of renin is about the same as for Bence-Jones proteins or about 5 000 less, that is 43 000 (Bence-Jones protein "F"), or 39 000 (Bence-Jones protein "M"). The first value is considered more correct as Bence-Jones protein "M" in turn-over studies in patients [6] appeared to be partially denatured.

The value of 43 000 agrees with the preliminary finding from 1964 (42 000 to 49 000) and with a statement by Peart [11] in a survey on renin from 1965 (about 50 000). Furthermore a similar result [8] (40 000) was obtained in an ultracentrifuge study of a purified renin preparation.

No change occurred in the determinations when decreasing amounts of renin were applied to the columns. Though adsorption of renin to the gel, resulting in too low estimates for the molecular weight, cannot be ruled out, this seems quite unlikely in view of the high degree of reproducibility of the results obtained and the symmetry of the elution curve with a recovery of about 100 %.

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